REVERSIBLE INHIBITION OF SWIMMING IN STOMOTOCA ATRA BY MESOGLEAL EXTRACTS OF SOME OTHER MEDUSAE

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When large numbers of the two predacious jellyfishes, *Stomotoca atra* and *Aequorea aequorea,* are collected in the same container of sea water, specimens of the smaller of the two hydromedusae, *S. atra,* sink to the bottom and remain there indefinitely. If, however, these non-swimming *Stomotoca* medusae are then isolated and placed in fresh sea water, they rise to the surface within 5 minutes and swim with their normal vigor. The unusual reaction of *Stomotoca atra* to the presence of *Aequorea* appears to result from a "tetanic" contraction of its velum. The causative agent is a heat-stable, dialyzable, non-toxic substance present in *Aequorea* and certain other medusae.

Materials and Methods

The organisms were collected at Friday Harbor, Washington, in the summer of 1962. The medusae were removed from the sea in a glass vessel, rather than in a net, in order to preserve the integrity of their delicate tissues. The animals were ordinarily used within four hours after capture. Fresh sea water was employed in most of the experiments. The temperature was kept at 13° C. by setting the experimental glass containers on a sea table of running sea water.

Results

1. Diffusion of an inhibitory substance from intact Aequorea aequorea and from isolated pieces of its mesoglea

In a typical experiment, a specimen of *A. aequorea* (60 mm. in diameter and 30 ml. in volume) was placed in a dish of 200 ml. sea water. The *Aequorea* was pulsating, the manubrium was closed, and the surface epithelium was not visibly broken. A single *Stomotoca* was then placed in the dish of sea water containing the *Aequorea.* The *Stomotoca* swam actively for about 6 minutes before any lessening of swimming could be noted. After 30 minutes, however, it was barely moving. By 45 minutes, it ceased swimming and assumed a contracted state.

Next, a piece of *Aequorea* mesoglea, free of epithelium and approximately 15 ml. in volume, was placed into 100 ml. of sea water containing two *Stomotoca* medusae. The swimming movements of the medusae were inhibited at 45 and 60 seconds, respectively; the animals were still inhibited 8 hours later.

These experiments show that (a) intact *Aequorea* medusae emit a substance which inhibits the swimming activities of *Stomotoca atra,* (b) the inhibitory sub-

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stance is present in a freely diffusible form in the mesoglea, and (c) this substance can slowly (but not readily) pass through an epithelial barrier.

2. Inhibition studies using mesogleal fluids

Aequorea mesogleal fluids, which were presumed to contain a high concentration of the inhibitory agent, were acquired by scraping off the dorsal epithelium, carving out and removing a conical piece of mesoglea, and allowing the mesogleal fluids to exude into the apex of the cone-shaped well.

Specimens of *Stomotoca* were inhibited from swimming within 10 to 15 seconds after being placed in a 1/10 dilution of this mesogleal fluid. Proportionally slower inhibitions, by 20 to 50 seconds, took place in all animals kept in a 1/20 dilution. At 1/40, some animals were inhibited at 30 seconds and others as late as 11.6 minutes. In addition, some of these inhibited animals partially recovered their swimming movements in the subsequent 4.3 minutes. At 1/100 dilutions, inhibitions started at 7.5 minutes. The latter animals usually recovered within 40 minutes while remaining in the same solution.

3. Preparation of large amounts of mesogleal fluid

Large portions of the mesoglea were excised free of epithelia and twisted in a clean cotton cloth until most of the fluid was expressed. Since *A. aequorea* is about 96% water (Hyman, 1940), the volume of fluid collected was nearly equal to the volume of mesoglea placed into the cloth. The expressed fluid, diluted with sea water, inhibited the swimming of *Stomotoca* at dilutions almost identical with those of the mesogleal fluid obtained from the cone-shaped cavity in Expt. 2.

It can be assumed that the concentration of the inhibitor in specimens of *Aequorea* of the same size is probably similar from animal to animal since dilutions of these expressed fluids consistently inhibited the swimming of *S. atra* within 20 to 50 seconds. (The time required for inhibition to occur varied somewhat with the size of *Stomotoca*, the smaller medusae being inhibited more quickly than the larger ones.) Thus, specific dilutions of the mesogleal fluids can represent reliable and quantitative expression of the relative amounts of the inhibitory agent. Since 1/20 dilutions consistently induced unequivocal and rapid inhibitions, this dilution was used as the testing concentration in all subsequent experiments.

4. Hydrogen ion concentration of the mesogleal fluid

The pH of the undiluted *Aequorea* mesogleal fluid was 7.6; that of the 1/20 dilution was 7.91, which is identical to the pH of sea water. Therefore, we can exclude a difference in hydrogen ion concentration as the cause of the inhibition.

5. Effects of heat and dialysis on the inhibitory properties of the mesogleal extract

Twenty-five ml. of the mesogleal fluid were heated for 15 minutes to 85° C. and then diluted 1:20. This diluted, heat-treated solution, like the untreated fluid, inhibited the swimming of *Stomotoca* in less than 30 seconds.

A 20-ml. aliquot of a fresh extract was dialyzed against 80 ml. of sea water. After 8 hours a portion of the water surrounding the bag was diluted to a volume which would give a theoretical concentration of 1/20 of the original fluid, assuming complete dialysis. This dilute solution inhibited two specimens of *Stomotoca* within 30 and 50 seconds, respectively. An equivalent of a 1/10 dilution inhibited the swimming of the medusae within 5 seconds.

The above experiments show that the active component of the mesogleal fluid is heat-stable and dialyzable—and, therefore, probably a small molecule. Accordingly, the inhibitory action is not likely due to any immunologically active macromolecule.

6. Origin of inhibitory substance

Aqueous extracts of the epithelia of *Aequorea* were prepared and tested for inhibitory activity as follows: First the bulk of the mesoglea of *Aequorea* was removed and extracted. Next, the remaining parts of the animal, which contained nearly all the cells and some mesoglea, were expressed through a cotton cloth. At 1/20 the mesoglea extract took 20 to 30 seconds to act, while the "cell" extract inhibited in less than 5 seconds. At 1/100 the mesoglea extract inhibited at 40 and 150 seconds; after 10 minutes, the same solution—with the same animals—was further diluted to 1/200 and the animals recovered by 6.1 minutes. In contrast, the extracts of *Aequorea* cells at 1/100 dilutions inhibited at 5 and 20 seconds; these inhibited animals did not recover by the subsequent 1:2 dilution. Furthermore, at 1/200 the mesogleal fluids had no visible inhibitory action, while the cell extracts inhibited at 25 and 40 seconds.

These experiments indicate that the inhibiting substance originates in the epithelial cellular areas of *Aequorea*, and that some then diffuses into the mesoglea and out of the animal. This observation suggests hitherto unexplored transport and storage functions for the mesoglea.

It is possible that part of the greater activity of the cell extract might be due to some nematocyst toxin released while the extract was prepared; this seems unlikely, however, because the inhibitor is found in the mesoglea which is free of epithelia and nematocysts.

7. Reversibility of the inhibition

Specimens of non-swimming *Stomotoca* found in the collection bucket with *Aequorea* medusae resumed swimming within minutes after being placed in fresh sea water, indicating that the inhibition was reversible. To test for reversibility, medusae that were inhibited by a 1/20 dilution of mesogleal extract for 10 minutes were placed in 600 ml. of fresh sea water. Within 75 seconds the medusae went from the contracted non-swimming state to a relaxed state. By 95 seconds, they pulsated occasionally. At 150 seconds the pulsations were forceful enough to cause slight swimming movements, and by 165 seconds the animals were swimming actively.

These general orders of events occurred with all animals, regardless of the length of time they were previously exposed to the inhibitor. Also, the shorter the

exposure to the inhibitor, the more quickly active swimming movements were resumed in fresh sea water. For example, animals exposed for 5 minutes recovered in two minutes, while those inhibited for 20 minutes recovered within 5–8 minutes. All medusae kept in the 1/20 diluted extracts for 12 hours swam actively within 11 minutes when placed in fresh sea water.

Furthermore, animals kept in the 1/20 diluted *Aequorea* extracts for 24 hours, although inhibited for most of that period, gradually overcame the inhibition and recovered their swimming movements without being placed in fresh sea water.

In addition, by establishing the reversibility of the inhibitions, these experiments indicate that under the conditions used, the inhibitor was not toxic.

8. Action of Aequorea mesogleal extracts on intact and dissected parts of Stomotoca atra

When a *Stomotoca* is placed in the diluted mesogleal extract (1/20) the mouth bends from side to side. Next, the velum and radial canal contract, the velum converting from a circular to a "square" shape with "corners" equidistant between the radial canals. This latter contraction is probably responsible for the cessation of the swimming movements.

The mesogleal extracts (1/20) had similar effects on freshly dissected parts of *Stomotoca*. The isolated mouth (with attached gonads) twisted from side to side. Isolated tentacles contracted, coiling and uncoiling like a spring. In a "quarter" of the umbrella, the single radial canal and the accompanying velum contracted. Finally, a ring of the isolated velum (5 cm. long) coiled immediately and remained in the coiled state as long as it was in the extracts. When this coil of velum was placed into fresh sea water, it elongated somewhat and began a series of regular contractions.

9. Specificity of response

Neither *Sarsia tubulosa, Sarsia flammea, Phialidium hemisphericum, Halistaura, Gonionemus, Probosidactyla*, nor the ctenophore, *Pleurobracia* (a prey of *Aequorea*), were inhibited by a 1/20 dilution of the *Aequorea* mesogleal fluids. The *Probosidactyla* medusae, however, did show some signs of "discontentment" after 30 minutes. Thus, of the animals tested, the inhibitory response was specific to *Stomotoca atra.*

10. Specificity of inhibitor source

Extracts of other medusae were tested for inhibitory activity. Since it was difficult to remove large amounts of clean mesoglea from these other forms, I used extracts of the whole animal. Of the medusae tested, only extracts of *Halistaura* were effective at 1/200 dilution (as was *A. aequorea*). *Sarsia tubulosa* extracts showed activity at 1/100 dilution, although the *Stomotoca* started to recover within a few minutes after the inhibition. Extracts of *Phialidium hemisphericum* were active both at 1/20 and 1/100 dilutions, although within an hour animals from the 1/200 experiment swam occasionally, while some from the 1/100 experiment were

swimming vigorously. Only extracts of *Stomotoca* itself had no inhibitory action. When placed in a 1/20 dilution of the whole *Stomotoca,* the test animals swam as vigorously as the controls throughout the 12-hour observational period.

Discussion

The small, two-tentacled, predacious jellyfish, *Stomotoca atra*—when exposed to extracts from other medusae—is reduced to a contracted state during which swimming ceases. This induced inhibition is markedly different from the relaxed state of *Stomotoca* observed during the transient cessation of swimming that follows its normal pulsating movements.

Not enough is known about the behavior of these organisms in nature to attribute any functions to this response as yet. At first I erroneously conjectured that since *Stomotoca atra* is a predator of other jellyfishes, the prey evolved a substance which essentially "narcotized" the predator. This is not plausible because even *Phialidium,* a common food of *Stomotoca,* contains this substance. Furthermore, it seems unlikely that in the ocean sufficient inhibitor would accumulate in the general area of these rapidly pulsating medusae to have any physiological importance.

Alternatively, the inhibitor might be considered a common metabolite of most medusae. Since the mouth of *Stomotoca* would be in close contact with the prey (the source of the inhibitor), then the mouth would be the first (and perhaps only) part of the *Stomotoca* to contract. And these contractions might aid *Stomotoca* in feeding by stimulating a suction-type peristalsis. The mouth was active in capturing and ingesting live prey even after I removed from *Stomotoca* its two tentacles or complete umbrella. Thus, this proposed chemical induction of a suction-type of feeding may be a useful adaptation by *Stomotoca* enabling it to ingest prey medusae without employing the manipulating actions of its tentacles.

This report shows that *Stomotoca atra* exhibits an unusual and specific response to extracts of other medusae. The nature of the inhibitory substance(s), the site(s) of its action, and the mechanism of its action invite further investigation.

I wish to thank Dr. Dixy Lee Ray, Dr. Demorest Davenport, and Dr. Paulo Sawaya for their encouragement of this research.

Summary

1. Specimens of *Stomotoca atra* ceased to swim when they were placed in the same container with *Aequorea aequorea* medusae.

2. This inhibition of swimming was also produced by pieces of the isolated mesoglea from *A. aequorea,* and by mesogleal extracts from the latter.

3. Methods for preparing large amounts of a "standard" mesogleal extract of *Aequorea* are described.

4. The inhibitory action of the fluids was not a result of change in pH.

5. The inhibitory agent from *Aequorea* mesogleal fluids was heat-stable and dialyzable.

6. Larger concentrations of the inhibitory substances were found in the non-mesogleal portions of *Aequorea*.

7. The inhibitions were completely reversible, even after 12 hours' constant exposure to a 1/20 mesogleal extract.

8. The mesogleal extracts affected individual parts of the dissected *S. atra*.

9. The swimming of no other medusae tested was affected by the mesogleal extracts of *Aequorea*.

10. Extracts from all medusae tested, except from *Stomotoca atra* itself, inhibited the swimming of the *S. atra*.

LITERATURE CITED

HYMAN, L. H., 1940. Observations and experiments on the physiology of medusae. *Biol. Bull.*, 79: 282–296.